

10-2009

# Pathogenicity of an emergent, ovine abortifacient *Campylobacter jejuni* clone orally inoculated into pregnant guinea pigs

Eric R. Burrough

*Iowa State University*, burrough@iastate.edu

Orhan Sahin

*Iowa State University*, osahin@iastate.edu

Paul J. Plummer


*Iowa State University*, pplummer@iastate.edu

Qijing Zhang

*Iowa State University*, zhang123@iastate.edu

Follow this and additional works at: [http://lib.dr.iastate.edu/vmpm\\_pubs](http://lib.dr.iastate.edu/vmpm_pubs)

Michael J. Yaeger

 Part of the [Iowa State University Commons](http://lib.dr.iastate.edu/vmpm_pubs) and [Laboratory Animal Medicine Commons](http://lib.dr.iastate.edu/vmpm_pubs), [Veterinary Microbiology and Immunobiology Commons](http://lib.dr.iastate.edu/vmpm_pubs), [Veterinary Pathology and Pathobiology Commons](http://lib.dr.iastate.edu/vmpm_pubs), and the [Veterinary Preventive Medicine, Epidemiology, and Public Health Commons](http://lib.dr.iastate.edu/vmpm_pubs)

The complete bibliographic information for this item can be found at [http://lib.dr.iastate.edu/vmpm\\_pubs/178](http://lib.dr.iastate.edu/vmpm_pubs/178). For information on how to cite this item, please visit <http://lib.dr.iastate.edu/howtocite.html>.

---

# Pathogenicity of an emergent, ovine abortifacient *Campylobacter jejuni* clone orally inoculated into pregnant guinea pigs

## Abstract

**Objective**—To compare pathogenicity of an emergent abortifacient *Campylobacter jejuni* (IA 3902) with that of reference strains after oral inoculation in pregnant guinea pigs.

**Animals**—58 pregnant guinea pigs.

**Procedures**—12 animals were challenged IP with *C jejuni* IA 3902 along with 5 sham-inoculated control animals to confirm abortifacient potential. Once pathogenicity was confirmed, challenge via oral inoculation was performed whereby 12 guinea pigs received IA 3902, 12 received *C jejuni* isolated from ovine feces (OF48), 12 received a fully sequenced human *C jejuni* isolate (NCTC 11168), and 5 were sham-inoculated control animals. After abortions, guinea pigs were euthanized; samples were collected for microbial culture, histologic examination, and immunohistochemical analysis.

**Results**—*C jejuni* IA 3902 induced abortion in all 12 animals following IP inoculation and 6 of 10 animals challenged orally. All 3 isolates colonized the intestines after oral inoculation, but only IA 3902 induced abortion. Evidence of infection existed for both IA 3902 and NCTC 11168; however, *C jejuni* was only recovered from fetoplacental units of animals inoculated with IA 3902. Immunohistochemical analysis localized *C jejuni* IA 3902 infection to subplacental trophoblasts, perivascular tissues, and phagocytes in the placental transitional zone.

**Conclusions and Clinical Relevance**—This study revealed that *C jejuni* IA 3902 was a unique, highly abortifacient strain with the ability to colonize the intestines, induce systemic infection, and cause abortion because of its affinity for the fetoplacental unit. Guinea pigs could be effectively used in the study of septic abortion after oral inoculation with this *Campylobacter* strain.

## Disciplines

Comparative and Laboratory Animal Medicine | Veterinary Microbiology and Immunobiology | Veterinary Pathology and Pathobiology | Veterinary Preventive Medicine, Epidemiology, and Public Health

## Comments

This article is published as Burrough, Eric R., Orhan Sahin, Paul J. Plummer, Qijing Zhang, and Michael J. Yaeger. "Pathogenicity of an emergent, ovine abortifacient *Campylobacter jejuni* clone orally inoculated into pregnant guinea pigs." *American journal of veterinary research* 70, no. 10 (2009): 1269-1276. doi: <https://doi.org/10.2460/ajvr.70.10.1269>. Posted with permission.

## Pathogenicity of an emergent, ovine abortifacient *Campylobacter jejuni* clone orally inoculated into pregnant guinea pigs

Eric R. Burrough, DVM; Orhan Sahin, DVM, PhD; Paul J. Plummer, DVM; Qijing Zhang, BVSc, PhD; Michael J. Yaeger, DVM, PhD

**Objective**—To compare pathogenicity of an emergent abortifacient *Campylobacter jejuni* (IA 3902) with that of reference strains after oral inoculation in pregnant guinea pigs.

**Animals**—58 pregnant guinea pigs.

**Procedures**—12 animals were challenged IP with *C jejuni* IA 3902 along with 5 sham-inoculated control animals to confirm abortifacient potential. Once pathogenicity was confirmed, challenge via oral inoculation was performed whereby 12 guinea pigs received IA 3902, 12 received *C jejuni* isolated from ovine feces (OF48), 12 received a fully sequenced human *C jejuni* isolate (NCTC 11168), and 5 were sham-inoculated control animals. After abortions, guinea pigs were euthanized; samples were collected for microbial culture, histologic examination, and immunohistochemical analysis.

**Results**—*C jejuni* IA 3902 induced abortion in all 12 animals following IP inoculation and 6 of 10 animals challenged orally. All 3 isolates colonized the intestines after oral inoculation, but only IA 3902 induced abortion. Evidence of infection existed for both IA 3902 and NCTC 11168; however, *C jejuni* was only recovered from fetoplacental units of animals inoculated with IA 3902. Immunohistochemical analysis localized *C jejuni* IA 3902 infection to sub-placental trophoblasts, perivascular tissues, and phagocytes in the placental transitional zone.

**Conclusions and Clinical Relevance**—This study revealed that *C jejuni* IA 3902 was a unique, highly abortifacient strain with the ability to colonize the intestines, induce systemic infection, and cause abortion because of its affinity for the fetoplacental unit. Guinea pigs could be effectively used in the study of septic abortion after oral inoculation with this *Campylobacter* strain. (*Am J Vet Res* 2009;70:1269–1276)

Abortions in sheep as a result of campylobacteriosis are common throughout the world, with regional variability in the species isolated and abortion rate.<sup>1</sup> In a 2001 survey<sup>2</sup> that spanned 22 states and included 72.3% of US sheep producers, campylobacteriosis was reported as the most common cause of infectious abortion in domestic flocks, with 53.7% of those reports confirmed by a veterinarian or diagnostic laboratory. Historically, *Campylobacter fetus* subsp *fetus* was the predominant isolate in sheep with campylobacteriosis, whereas *Campylobacter jejuni* and *Campylobacter coli* accounted for a smaller percentage.<sup>3–6</sup> However, 2 US studies<sup>7,8</sup> have indicated a dramatic shift in this relationship, with *C jejuni* becoming the predominant species causing abortion in sheep. Abortion-causing

ABBREVIATION	
MH	Mueller-Hinton

isolates of *C fetus* subsp *fetus* and *C jejuni* have considerable heterogeneity.<sup>9–11</sup> Analysis by use of pulsed-field gel electrophoresis has revealed a major shift in the distribution of isolates. An initial study<sup>8</sup> of *C jejuni* isolates from aborting ewes in Iowa flocks revealed a preponderance of a single clone despite recovery from multiple Iowa farms during multiple lambing seasons.<sup>8</sup> Expansion of this evaluation confirmed that this single clone of tetracycline-resistant *C jejuni* was the predominant *Campylobacter* strain isolated from abortions of sheep in South Dakota, Idaho, California, Oregon, and Nevada.<sup>8</sup> These findings strongly suggest that a highly abortifacient strain of *C jejuni* (named IA 3902) has emerged that is now a leading cause of abortion outbreaks in sheep in the United States.

Intraperitoneal inoculation of pregnant guinea pigs has been used as a method to evaluate the pathogenicity of *Campylobacter* spp<sup>12,13</sup> and to assess the efficacy of vaccines.<sup>14–16</sup> Their small size, ease of housing, and relatively short duration of gestation make guinea pigs a desirable animal for use in studying *Campylobacter*-

Received November 5, 2008.

Accepted December 8, 2008.

From the Departments of Veterinary Pathology (Burrough, Yaeger) and Veterinary Microbiology and Preventive Medicine (Sahin, Plummer, Zhang), College of Veterinary Medicine, Iowa State University, Ames, IA 50011.

Supported by a grant from the Iowa Livestock Health Advisory Council. The authors thank Deborah Moore for assistance with the immunohistochemical analysis.

Address correspondence to Dr. Yaeger ([myaeger@iastate.edu](mailto:myaeger@iastate.edu)).



induced abortion, particularly when compared with the use of sheep or goats, which are the species most commonly affected in nature. However, an IP technique is not totally appropriate for use in evaluating the pathogenesis of septic abortions attributable to *C jejuni* because it bypasses critical initial steps in the abortion process, including intestinal colonization and bacterial invasion. Currently, there are limited data regarding oral inoculation of *C jejuni* in guinea pigs. In another study,<sup>17</sup> oral inoculation of 4 pathogenic isolates of *C jejuni* obtained from the intestines of humans failed to induce abortion in pregnant guinea pigs. We hypothesize that this was attributable to differences in virulence among *C jejuni* isolates and that highly abortifacient clinical isolates, such as *C jejuni* IA 3902, should be effective in inducing abortion after oral inoculation.

In the study reported here, we compared *C jejuni* IA 3902 with an isolate obtained from the feces of sheep (*C jejuni* OF48) and an isolate obtained from humans (*C jejuni* NCTC 11168) to determine their ability to induce abortion after oral inoculation in pregnant guinea pigs. In all guinea pigs that aborted, immunohistochemical analysis was used to identify and localize organisms within placental tissues to provide insights into mechanisms involved in *Campylobacter*-induced abortion.

## Materials and Methods

**Animals**—Fifty-eight pregnant (approx 2 to 3 weeks of gestation) Hartley guinea pigs were obtained from a commercial source<sup>a</sup> for use in the study. Seventeen guinea pigs were used in a preliminary IP challenge experiment. Mean weight at time of arrival for these guinea pigs was 865 g. Subsequently, 41 pregnant guinea pigs were used in an oral challenge experiment. Mean weight at time of arrival for these guinea pigs was 797 g. At arrival, a rectal swab specimen was obtained from each guinea pig, placed in transfer medium, and plated for *Campylobacter* culture.

Guinea pigs were housed in standard metal cages with wood chip bedding and fed a pelleted commercial diet formulated for guinea pigs. Animals were housed in groups (4 or 5 guinea pigs/cage for the IP challenge exposure and 3 or 4 guinea pigs/cage for the oral challenge exposure) and allowed to acclimate to their environment for 7 days prior to inoculation. During this time, weights were recorded daily and guinea pigs were marked with black hair dye to provide unique identification for each animal. For the oral challenge exposure, treatment groups were housed in separate banks of cages; strict hygienic procedures were used between handling of groups to prevent cross-contamination. All procedures were approved by the Institutional Animal Care and Use Committee at Iowa State University.

***Campylobacter* strains**—*Campylobacter jejuni* isolates were used in challenge exposures. The IA 3902 isolate was cultured from an aborted ovine fetus. This tetracycline-resistant strain of *C jejuni* is the predominant genetic clone associated with abortion in sheep, as determined by pulsed-field gel electrophoresis, multilocus sequence typing, and *cmp* gene sequence typing.<sup>8</sup> The OF48 isolate was cultured from feces of a sheep

that did not have clinical signs (abortion or diarrhea). The NCTC 11168 isolate<sup>b</sup> is from humans and has been completely sequenced.<sup>18</sup> Fresh bacterial cultures were obtained following 24 hours of growth on MH agar in anaerobic jars under microaerobic conditions (5% oxygen, 10% carbon dioxide, and 85% nitrogen) at 42°C. These cultures were collected and placed in MH broth, diluted to desired concentrations on the basis of optical density, and then used as inocula in the challenge experiments. The final number of organisms in each suspension was determined by counting the number of viable CFUs.

**IP challenge exposure**—Two groups of guinea pigs were used in a preliminary experiment. The first group (which comprised 5 guinea pigs) served as sham-inoculated control animals and were inoculated IP with 1.0 mL of MH broth IP, whereas the second group (which comprised 12 guinea pigs) was inoculated IP with 1.0 mL of  $1.5 \times 10^5$  CFUs of *C jejuni* IA 3902/mL of MH broth. This concentration was chosen on the basis of reports<sup>12,13</sup> in which pregnant guinea pigs were challenge exposed with *C jejuni* via IP inoculation. Guinea pigs in both groups were inoculated by use of 1-mL tuberculin syringes with a 26-gauge, 3/8-inch needle.

**Oral inoculation**—Four groups of guinea pigs were used in the oral challenge exposure experiment. The first group ( $n = 5$  guinea pigs) served as sham-inoculated control animals and received 1.0 mL of MH broth. The second group ( $n = 12$  guinea pigs) received 1.0 mL of  $5.5 \times 10^8$  CFUs of *C jejuni* OF48/mL of MH broth. The third group ( $n = 12$  guinea pigs) received 1.0 mL of  $5.9 \times 10^8$  CFUs of *C jejuni* NCTC 11168/mL of MH broth. The fourth group ( $n = 12$  guinea pigs) received 1.0 mL of  $6.0 \times 10^8$  CFUs of *C jejuni* IA 3902/mL of MH broth. These doses were chosen to approximate those used in other experiments in which *C jejuni* was administered orally to pregnant ferrets<sup>19</sup> and guinea pigs.<sup>17</sup> Guinea pigs in all 4 groups were inoculated orally via a curved, 18-gauge, 3-inch, stainless-steel feeding needle. Food was withheld from the guinea pigs for 12 hours prior to inoculation to diminish the amount of food retained in the oral cavity.

**Monitoring, euthanasia, necropsy, and collection of samples**—After inoculation, guinea pigs were weighed daily by use of a digital gram scale and were observed at least twice daily for signs of abortion or impending abortion. These signs included vaginal bleeding, expelled fetuses or fetal membranes, and sudden substantial weight loss ( $> 10\%$  in 24 hours). Once a guinea pig had aborted or an impending abortion was identified, that animal was euthanized via IP injection of sodium pentobarbital (approx 150 mg/kg) and immediately necropsied. All guinea pigs that had not aborted were euthanized 21 days after inoculation and necropsied.

Necropsy of guinea pigs involved inspection for gross lesions and collection of samples for bacterial culture and histologic examination. Rectal swab specimens for *Campylobacter* culture were obtained by use of sterile minitip cotton swabs<sup>c</sup> immediately after euthanasia. Other samples harvested for *Campylobacter* cul-



ture included heart blood, bile, and uterine tissue from each dam and placenta, liver, and lung tissues from each fetus. Pooled fetal stomach contents were collected only during the oral challenge portion of the study. Heart blood, bile, and fetal stomach contents were collected by use of a sterile tuberculin syringe with a 26-gauge, 3/8-inch needle. Samples of uterine, placental, and pooled fetal liver and lung tissues were placed in separate sterile plastic bags<sup>d</sup> (IP challenge) or separate sterile Petri plates (oral challenge). All samples were refrigerated until immediately prior to culture, with bacterial culture performed on most samples on the day of collection.

Samples collected for histologic examination included liver, gallbladder, and uterine tissues for each dam and placenta, liver, and lung tissues for each fetus. Samples obtained for histologic examination were placed in neutral-buffered 10% formalin for 24 hours, embedded in paraffin, and processed routinely for H&E staining.

**Campylobacter culture and semiquantitative enumeration of *C. jejuni* from necropsy samples**—A few drops of each fluid sample (blood, bile, or fetal stomach contents) were placed directly onto culture media and streaked by use of sterile cotton swabs. Rectal swab specimens were streaked directly onto culture media. Uterine tissues, pooled fetal liver and lung tissues, and placental tissues were minced with a scalpel or scissors; then, swab specimens were obtained from the minced tissues by use of a sterile cotton swab and streaked onto culture media. All samples were spread onto MH agar containing a *Campylobacter* selective supplement<sup>e</sup> (polymyxin B, rifampicin, trimethoprim, and cycloheximide) and a *Campylobacter* growth supplement<sup>f</sup> (sodium pyruvate, sodium metabisulfite, and ferrous sulfate) and were incubated for 48 hours in anaerobic jars under microaerobic conditions at 42°C. After incubation, *Campylobacter*-like colonies on each plate were counted to determine the number of CFUs in each sample.

**Histologic scoring of uterine lesions**—Endometrial inflammation was evaluated by calculating the mean number of leukocytes in 10 hpfs (40X magnification). Uterine lesions were scored in each hpf by use of the following criteria: 0 = inflammatory infiltrate was not identified, 1 = < 10 leukocytes/hpf, 2 = 10 to 20 leukocytes/hpf, and 3 = > 20 leukocytes/hpf. An additional 0.5 points was added to the score when the inflammation extended into the underlying myometrium.

**Immunohistochemical analysis**—Placental samples were collected at necropsy and embedded in paraffin, as described previously. Tissues were sectioned at a thickness of 3 µm, mounted on aminoalkylsilane-coated glass slides, and placed in an oven at 56°C for 2 hours. Sections were routinely deparaffinized in xylene and rehydrated in graded alcohol solutions and water baths. Endogenous peroxidase inhibition was achieved by immersion (2 immersions; 10 min/immersion) in baths of 3% H<sub>2</sub>O<sub>2</sub> in water. Sections were incubated with 0.1% protease in a Tris buffer (pH, 7.6) at 37°C for 15 minutes. Slides were rinsed 3 times in PBS solution and then were placed in an automated cell stain-

ing system.<sup>g</sup> To inhibit nonspecific binding, sections were incubated in 10% neutral goat serum at 22°C for 20 minutes. The primary antibody, which was directed against the major outer membrane protein of *C. jejuni*, was prepared as described in another study.<sup>20</sup> This antibody was used at a dilution of 1:300; slides were incubated at 22°C for 60 minutes followed by rinsing in a bath of PBS solution for 5 minutes. A commercially available biotinylated secondary antibody<sup>h</sup> was used at a dilution of 1:80; slides were incubated at 22°C for 15 minutes followed by rinsing in a bath of PBS solution for 5 minutes. Sections were then incubated with horse radish peroxidase–streptavidin conjugate<sup>i</sup> at 22°C for 15 minutes followed by rinsing in a bath of PBS solution for 5 minutes. The final reaction was developed by use of a commercial chromogen.<sup>j</sup> Sections were rinsed and routinely counterstained with Shandon Harris hematoxylin<sup>k</sup> and Scott's tap water. Sections were dehydrated through graded alcohol and xylene solutions prior to mounting. Positive control samples were obtained from paraffin blocks of ovine placental tissues that had positive results when cultured for *C. jejuni* IA 3902. Negative control samples consisted of sections from culture-negative tissues obtained from nonaborting guinea pigs inoculated with *C. jejuni* IA 3902 and from sham-inoculated control guinea pigs.

**Statistical analysis**—A commercial statistical software package<sup>l</sup> was used to perform 1-way ANOVAs to detect differences among groups. Abortion rates, mean uterine scores, necrosuppurative placentitis, and random necrotizing hepatitis were compared. Results were considered significant at values of  $P \leq 0.05$ .

## Results

**IP challenge exposure**—All rectal swab specimens collected before challenge exposure had negative results when cultured for *Campylobacter* spp. Ten of 12 guinea pigs inoculated with IA 3902 aborted, with 8 guinea pigs aborting 2 or 3 days after inoculation and the remaining 2 guinea pigs aborting 8 days after inoculation. Necropsy revealed that the 2 guinea pigs that did not abort were not pregnant; 1 of these guinea pigs had a teratoma of the left ovary. For the control group, none of the 5 guinea pigs aborted and necropsy revealed that all 5 were pregnant.

Calculation of the abortion rate excluded the 2 guinea pigs that were not pregnant. Thus, the mean abortion rate for guinea pigs inoculated IP with IA 3902 (100%) was significantly ( $P < 0.001$ ) higher than the mean abortion rate for the control guinea pigs (0%).

**Oral challenge exposure**—All rectal swab specimens collected before challenge exposure had negative results when cultured for *Campylobacter* spp. Six of 12 guinea pigs inoculated with IA 3902 aborted; 4 guinea pigs aborted between 4 and 6 days after inoculation, and the other 2 guinea pigs aborted between 7 and 10 days after inoculation. None of the guinea pigs in the remaining groups aborted. Necropsy revealed that 2 guinea pigs in each of the IA 3902, NCTC 11168, and OF48 groups and 1 guinea pig in the control group were not pregnant; we determined that these guinea pigs had



not been pregnant during the study on the basis of a lack of appreciable weight gain. These guinea pigs were excluded from the calculations for abortion rate.

Mean abortion rates differed significantly ( $P < 0.001$ ) among groups (Table 1). The mean abortion rate for guinea pigs inoculated with IA 3902 (6/10) was significantly ( $P < 0.001$ ) higher than the rate for any of the other 3 groups (0/10, 0/10, and 0/4 for guinea pigs inoculated with NCTC 11168, OF48, or the sham inoculation, respectively). In addition, necropsy revealed that 1 of the 4 pregnant, nonaborting guinea pigs from the IA 3902 group had histologic and bacteriologic evidence of fetal infection.

Gross liver lesions consisted of multiple, variably sized, round, white foci; they were identified in 9 guinea pigs inoculated with IA 3902 and 3 guinea pigs inoculated with NCTC 11168. The predominant histopathologic liver lesion was random, multifocal to coalescing hepatitis, which was detected in decreasing frequency in the IA 3902 (11/12), NCTC 11168 (8/12), and OF48 (4/12) groups; this lesion was not detected in the control group (0/4). Inflammatory foci were variably sized, and the associated infiltrate differed depending on the interval between challenge exposure and necropsy. In guinea pigs that aborted within a few days after challenge exposure, inflammatory foci were primarily neutrophilic. As the interval between challenge exposure and abortion increased, the infiltrate became dominated by lymphocytes, plasma cells, and macrophages.

Mean incidence of multifocal random hepatitis differed significantly ( $P < 0.001$ ) among groups. The mean incidence of random hepatitis was significantly ( $P < 0.001$ ) higher in the IA 3902 group, compared with the mean incidence for the other 3 groups. The mean incidence of random hepatitis was significantly ( $P = 0.004$ ) higher in the NCTC 11168 group, compared with the mean incidence for the control group, but did not differ significantly ( $P = 0.092$ ) from the mean incidence for the OF48 group. Mean incidence of random hepatitis for the OF48 group did not differ significantly ( $P = 0.136$ ) from the mean incidence for the control group.

Mild periportal infiltrates of lymphocytes and plasma cells were detected in most of the samples from all groups (Table 1). Additionally, congestion or edema of the gallbladder was evident in 5 guinea pigs of the IA 3902 group, 5 guinea pigs of the NCTC 11168 group, 3 guinea pigs of the OF48 group, and 1 guinea pig of the control group.

Uterine lesions were variable and consisted of suppurative endometritis, metritis, and hemorrhage of varying severity. Uterine edema was evident in guinea pigs from all the IA 3902, NCTC 11168, and OF48 groups but was most severe in those from the IA 3902 group. In general, uterine inflammatory scores were highest in guinea pigs inoculated with IA 3902 (mean, 2.3; range, 1.0 to 3.5), with lower scores in a few guinea pigs of the OF48 (mean, 0.4; range, 1.0 to 2.5) and control (mean, 0.5; range, 1.0 to 1.5) groups and no metritis detected in the NCTC 11168 group.

Mean uterine scores differed significantly ( $P < 0.001$ ) among groups (Table 1). Mean uterine score for the IA 3902 group was significantly ( $P < 0.001$ ) higher than the mean uterine score of any of the other 3 groups, whereas the mean uterine scores for the NCTC 11168 and OF48 groups did not differ significantly ( $P = 0.294$  and  $P = 0.792$ , respectively) from the mean uterine score for the control group.

Microscopic placental lesions consisted of a combination of hemorrhage, suppurative inflammation, and necrosis (Figure 1). Placental lesions were evident in 8 guinea pigs inoculated with IA 3902 (including all 6 that aborted) and in 1 guinea pig inoculated with NCTC 11168. These changes were detected in both the maternal and fetal portions of the placenta, with severity of the lesions increasing with an increase in the number of days after inoculation.

Mean incidence of necrosuppurative placentitis differed significantly ( $P < 0.001$ ) among groups (Table 1). Mean incidence of placentitis in the IA 3902 group (8/10) was significantly ( $P < 0.001$ ) higher than the mean incidence for the NCTC 11168 (1/10), OF48 (0/10), and control (0/4) groups; mean incidence for the NCTC 11168 and OF48 groups did not differ significantly ( $P = 0.569$  and  $P = 1.000$ , respectively), compared with the mean incidence for the control group.

High numbers of *C. jejuni* were cultured from 7 of 10 placental samples, 6 of 10 fetal liver or lung samples, and 6 of 10 uterine samples in the IA 3902 group. Moderate numbers of *C. jejuni* were cultured from 10 of 12 rectal swab specimens in the IA 3902 group, and low numbers of *C. jejuni* were generally cultured from 5 of 12 heart blood samples, 4 of 9 fetal stomach contents samples, and 2 of 12 bile samples in the IA 3902 group. Low to moderate numbers of *C. jejuni* were cultured from 9 of 12 rectal swab specimens from the NCTC 11168 group and 6 of 12 rectal swab specimens from the OF48 group. *Campylobacter jejuni* was not cultured

Table 1—Summary of strain-related differences in abortion rate and histopathologic lesions in guinea pigs after oral inoculation with *Campylobacter jejuni*.

Inoculum	Dose	Aborted*	Multifocal random hepatitis*	Necrosuppurative placentitis*	Mean uterine inflammatory score†
Sham	MH broth only	0/4	0/5	0/4	0.5 ± 0.4
OF48	5.5 × 10 <sup>8</sup> CFUs	0/10	4/12	0/9‡	0.4 ± 0.3
NCTC 11168	5.9 × 10 <sup>8</sup> CFUs	0/10	8/12	1/10	0 ± 0.3
IA 3902	6.0 × 10 <sup>8</sup> CFUs	6/10	11/12	8/10	2.3 ± 0.3

\*Represents the number of guinea pigs with this characteristic/number of guinea pigs inoculated. †Score ranged from 0 (inflammatory infiltrate not identified) to 3.5 (> 20 leukocytes/hpf and the inflammation extended into the underlying myometrium). ‡One guinea pig gave birth before the end of the study, and a placental sample was not obtained.



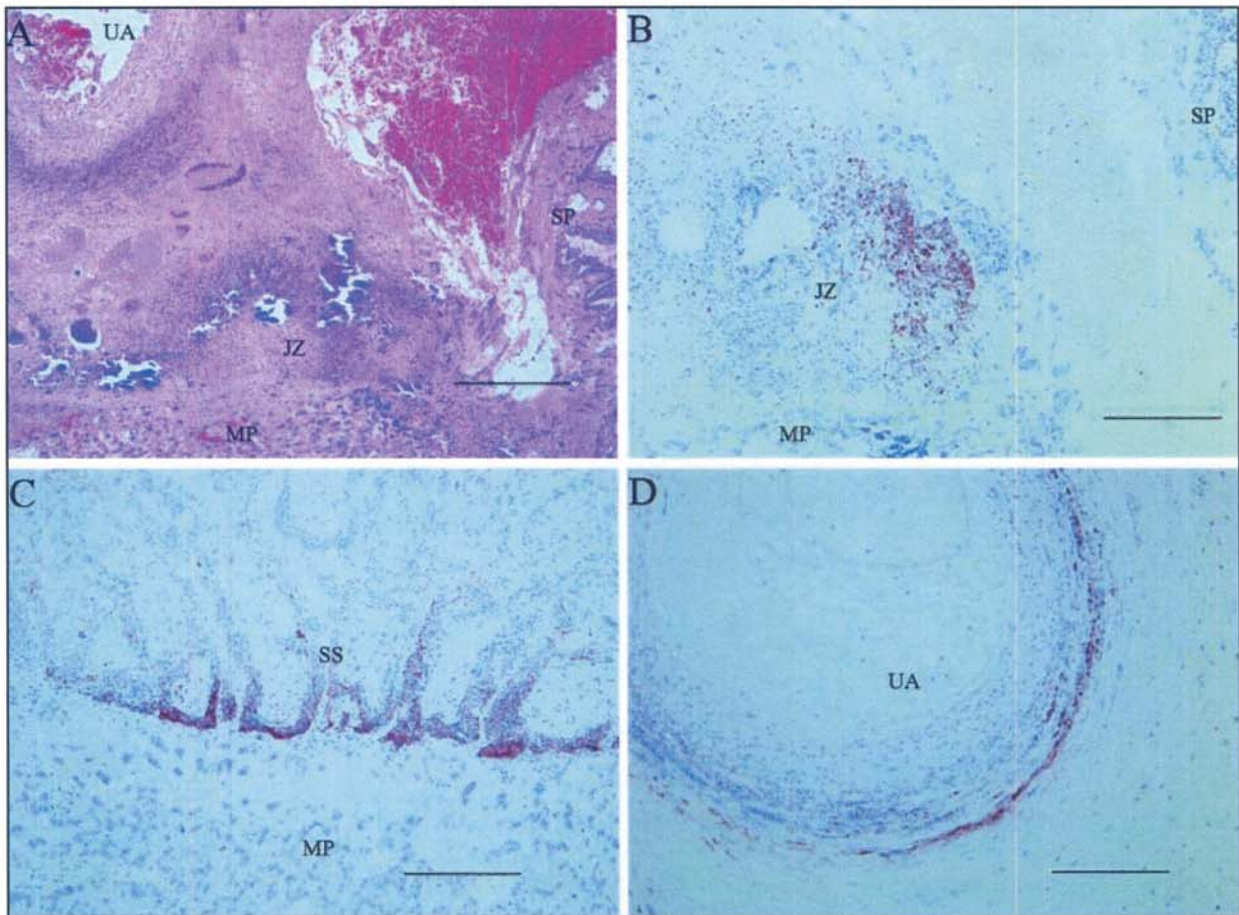


Figure 1—Photomicrographs of sections of placental tissues obtained from guinea pigs inoculated with *Campylobacter jejuni* IA 3902. A—Notice the necrosuppurative placentitis and hemorrhage at the junctional zone (JZ) between the subplacenta (SP) and main placenta (MP) and marked vasculitis of the adjacent uteroplacental artery (UA). H&E stain; bar = 200  $\mu$ m. B—Photomicrograph of the same section of placental tissue in panel A after immunohistochemical staining for *C. jejuni*. Notice the *C. jejuni* organisms (red stain) located within inflammatory cells and in extracellular spaces in the junctional zone. Primary antibody was directed against the major outer membrane protein of *C. jejuni*, followed by a biotinylated secondary antibody and incubation with horse radish peroxidase–streptavidin conjugate and developed by use of a commercial chromogen. Counterstained with Shandon Harris hematoxylin and Scott's tap water; bar = 200  $\mu$ m. C—Photomicrograph of a section of guinea pig placental tissue after immunohistochemical staining for *C. jejuni*. Notice the *C. jejuni* organisms (red stain) within the trophoblasts and syncytial streamers (SS) of the subplacenta. Bar = 200  $\mu$ m. D—Photomicrograph of a uteroplacental artery (UA) from a guinea pig that aborted 8 days after inoculation. Notice the marked vasculitis and abundant perivascular *C. jejuni* organisms (red stain). Immunohistochemical stain for *C. jejuni*; bar = 200  $\mu$ m.

from any other tissues in these groups or from any samples collected from the control group. The percentage of samples with positive results for culture of *C. jejuni* were summarized (Figure 2). The source and number of CFUs for bacterial cultures of guinea pigs inoculated with IA 3902 were also summarized (Table 2).

**Immunohistochemical analysis**—Immunohistochemical analysis for *C. jejuni* was performed on all available placental tissues from guinea pigs that aborted during the study. Staining for *C. jejuni* was not detected in any negative control samples or culture-negative aborted tissues. In guinea pigs inoculated IP with IA 3902, staining for *C. jejuni* was evident in 5 of 6 culture-positive samples (placental tissues or fetal tissues when placental samples were not available for culture). In guinea pigs inoculated orally with IA 3902, staining for *C. jejuni* was evident in 5 of 7 culture-positive samples. *Campylobacter jejuni* organisms were identified within the cytoplasm of subplacental trophoblasts, within

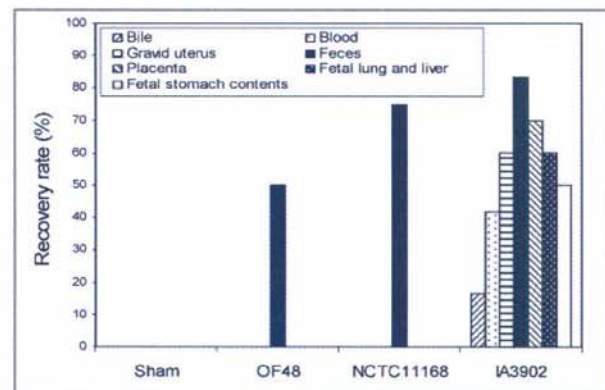


Figure 2—Percentage of samples with positive results when cultured for *C. jejuni*. Samples were obtained during necropsy of guinea pigs after oral inoculation with *C. jejuni* strain IA 3902, NCTC 11168, or OF48 or a sham inoculum. Necropsies were performed at the time of abortion or at 21 days after inoculation in guinea pigs that did not abort.



Table 2—Sample source and semiquantitative recovery of *C jejuni* cultured from samples obtained during necropsy of guinea pigs inoculated with IA 3902.

Sample source	No. of culture-positive samples*	Low recovery (< 50 CFUs)†	Moderate recovery (50 to 1,000 CFUs)†	High recovery (> 1,000 CFUs)†
Blood	5/12	3/5	2/5	0/5
Bile	2/12	0/2	1/2	1/2
Feces	10/12	2/10	6/10	2/10
Fetal stomach contents (pooled)*	4/8†	2/4	1/4	1/4
Gravid uterus	6/10	0/6	1/6	5/6
Fetal liver or lung (pooled)	6/10	1/6	0/6	5/6
Placenta	7/10	0/7	0/7	7/7

Values reported represent number of samples with positive culture results/number of samples tested.

†Results reported represent number of samples within that category/number of samples with positive culture results. ‡Fetal stomach contents were only obtained for 8 of 10 guinea pigs.

Pooled = Samples for all fetuses of a specific guinea pig were pooled for microbial culture.

phagocytes, and in extracellular spaces surrounding trophoblasts in areas of placentitis and necrosis (Figure 1). Organisms were most prevalent at the periphery of the subplacenta within the junctional zone. Multifocal intracellular organisms were also detected within the deeper portions of the endometrium and myometrium and surrounding the uteroplacental arteries; however, this distribution was limited to samples from guinea pigs that aborted at longer intervals after inoculation.

## Discussion

Campylobacteriosis has been described as the most common cause of infectious abortion in sheep in the United States,<sup>2</sup> and *C jejuni* has become the predominant species.<sup>7,8</sup> Pulsed-field gel electrophoresis, multilocus sequence typing, and *cmp* gene sequence typing of *C jejuni* isolates from multiple farms in multiple states during multiple lambing seasons have revealed that most of these isolates are of a single clone, which is represented by *C jejuni* IA 3902.<sup>8</sup> In the guinea pigs of the study reported here, we identified several unique features of *C jejuni* IA 3902, when compared with those of a sheep commensal fecal isolate (*C jejuni* OF48) and a fully sequenced human isolate (*C jejuni* NCTC 11168).

*Campylobacter jejuni* IA 3902 was uniquely proficient in its ability to induce abortion. In the preliminary experiment with IP challenge exposure, which was intended to verify the abortifacient potential of this isolate, all of the pregnant guinea pigs aborted. After oral inoculation, *C jejuni* IA 3902 was the only isolate that induced abortion (6/10 pregnant guinea pigs aborted). Had the study been extended a few additional days, the abortion rate after oral inoculation would likely have been even higher for IA 3902 because placentitis and fetal infection were identified in 1 guinea pig that had not yet aborted when the study was terminated. This is in contrast to results of a study<sup>17</sup> in which 4 pathogenic human isolates of *C jejuni* were evaluated in pregnant guinea pigs (abortion rates for those isolates ranged from 0% to 67% following IP inoculation and 0% following oral inoculation). To our knowledge, the study reported here is the first to reveal that oral inoculation of *C jejuni* can be effective in inducing abortion in pregnant guinea pigs. These results indicated that *C jejuni*

IA 3902 was highly abortifacient and that pregnant guinea pigs were an effective method for evaluating the pathogenicity of this isolate for a natural route of infection (oral) or after IP inoculation.

The pathogenesis of *Campylobacter* spp that induce abortion when acquired by a nonvenereal route of transmission entails oral exposure, intestinal colonization, bacterial invasion, bacteremia, and infection of the fetoplacental unit.<sup>1,21–23</sup> Sheep appear to be at increased risk for developing campylobacteriosis because healthy sheep often harbor *Campylobacter* spp within the intestines and gallbladder.<sup>1,24</sup> In a recent study,<sup>25</sup> *Campylobacter* spp were identified in 49.5% of intestinal, gallbladder, and fecal samples obtained from healthy sheep. The pathogenic mechanisms used by *C jejuni* in the evasion of the physical and immunologic barriers in the intestines have been extensively studied and have been described elsewhere.<sup>26,27</sup> In the study reported here, semiquantitative cultures of fecal samples revealed that all 3 strains of *Campylobacter* organisms were capable of colonizing the intestinal tract after oral administration, with *C jejuni* IA 3902 cultured in the greatest number of samples (10/12 [83%]) collected during necropsy, followed by *C jejuni* NCTC 11168 (9/12 [75%]) and OF48 (6/12 [50%]).

Once the intestines have been colonized, abortifacient *Campylobacter* spp must breach the intestinal epithelium and induce bacteremia. Bacteremia attributable to *C jejuni* has been described,<sup>28–30</sup> and results of 1 study<sup>31</sup> indicated that certain strains of *C jejuni* have an enhanced ability to induce bacteremia. Because of the stress associated with blood collection and the limited blood volume that can be harvested from guinea pigs on a daily basis, the magnitude and duration of bacteremia were not directly assessed in the present study, and an indirect gauge of systemic infection was used to estimate the incidence of bacteremia. Acute hepatitis has been reported as a sequela to bacteremia attributable to *C jejuni* in humans,<sup>32</sup> and liver biopsy specimens from a patient with acute hepatitis and concurrent enteritis attributable to *C jejuni* revealed nonspecific reactive hepatitis with focal necrosis.<sup>33</sup> Additionally, multifocal random hepatitis is a consistent necropsy finding secondary to bacteremia or septicemia for numerous organisms, including host-adapted *Salmonella* spp<sup>34</sup> such as *Salmonella enterica* serovar Dublin in cattle



and *Salmonella enterica* serovar choleraesuis in swine. In the study reported here, the identification of multifocal, random hepatitis was used as indirect evidence of systemic infection. This lesion was identified in 23 of 36 orally inoculated guinea pigs, including 11 of 12 guinea pigs inoculated with *C jejuni* IA 3902 and 8 of 12 guinea pigs inoculated with *C jejuni* NCTC 11168. The significantly higher rate of hepatitis in both groups indicated that each strain likely induced a degree of bacteremia, and it therefore seems likely that the differences in abortion rate were attributable to a mechanism other than the ability of these organisms to breach the intestinal epithelium and induce bacteremia.

Predilection for the fetoplacental environment is a common attribute of abortifacient agents, and it appears that the greatest difference between *C jejuni* IA 3902 and the other evaluated isolates was its affinity for the fetoplacental unit. All 3 isolates were able to colonize the intestines, and there was evidence that both *C jejuni* IA 3902 and *C jejuni* NCTC 11168 induced a substantial bacteremia after oral inoculation, yet only IA 3902 appeared to be able to colonize the placenta, infect trophoblasts, and induce abortion. Guinea pigs inoculated with *C jejuni* IA 3902 consistently developed endometritis and placentitis with high numbers of *C jejuni* (> 1,000 CFUs) recovered from uterine, placental, and pooled fetal tissues, all of which provided evidence for an apparent tropism of IA 3902 for the fetoplacental unit.

Evaluation of our results indicated that immunohistochemical analysis was less sensitive than microbial culture as a means of detecting *C jejuni* in tissues, which was consistent with results in other studies.<sup>35,36</sup> However, immunohistochemical analysis was extremely useful in characterizing the distribution of organisms in placental tissues. *Campylobacter jejuni* IA 3902 appeared to localize at the periphery of the subplacenta near its junction with the main placenta early during the course of infection and was evident within inflammatory cells and syncytiotrophoblasts of the subplacenta. This localization appears logical because this is the site of placental vascular invasion<sup>37</sup> and would be the first location reached during episodes of bacteremia. Additionally, guinea pigs infected for longer periods before they aborted often had immunohistochemical evidence of abundant pericellular and intracytoplasmic organisms associated with the periarterial trophoblasts in the decidua. These data suggested that trophoblasts of subplacental origin were preferentially infected, whereas those of the main placenta were spared. It is worth mentioning that there are considerable differences between the placentation of guinea pigs and ewes. Guinea pigs have a discoid, labyrinthine, hemochorial placenta with extensive trophoblast invasion of the decidua and uterine arterial walls.<sup>38,39</sup> In contrast, sheep have a cotyledonary, epitheliochorial placenta and lack trophoblast invasion of uterine blood vessels.<sup>39</sup> Although there are noticeable differences in placentation between these 2 species, our immunohistochemical findings of organisms within trophoblasts, leukocytes, and perivascular tissues were consistent with those in another study<sup>40</sup> of placentas from aborting sheep infected with *C jejuni*. Together, these findings suggest that pathogenic

*C jejuni* strains are capable of infecting trophoblasts regardless of placentation type, and this provides further supporting evidence for the appropriateness of the use of pregnant guinea pigs as a method for evaluation of *Campylobacter*-induced abortion. To the authors' knowledge, the information reported here was the first description of the localization and distribution of *Campylobacter* spp within the placenta of aborting guinea pigs.

In this study, we determined that *C jejuni* IA 3902 was a unique, highly abortifacient strain with the ability to colonize the intestines, cause systemic infection, and induce abortion as a result of its affinity for the fetoplacental unit. Although we confirmed the highly pathogenic nature of IA 3902 in pregnant guinea pigs, additional *in vivo* studies in sheep are required to determine the degree of virulence in pregnant ewes. *Campylobacter jejuni* IA 3902 appears to be a valuable strain for studying the pathogenesis of *Campylobacter*-induced abortion. To facilitate the study of pathogenic mechanisms, the genome of IA 3902 is being sequenced by our laboratory group, and we will compare it with a fully sequenced, nonabortifacient strain (NCTC 11168) to attempt to identify unique virulence traits of this abortifacient clone. Results also revealed that pregnant guinea pigs were useful for studying the pathogenesis of abortion attributable to this unique *Campylobacter* strain. Pregnant guinea pigs have been used to assess the efficacy of vaccines against *C fetus*,<sup>14-16</sup> and given the recent shift from *C fetus* to predominantly *C jejuni* causing abortions in sheep,<sup>8</sup> there is an urgent need to explore the development of a vaccine against *C jejuni* for the control of this emergent, highly pathogenic strain.

- a. Elm Hill Labs, Chelmsford, Mass.
- b. ATCC 700819, American Type Culture Collection, Manassas, Va.
- c. BBL CultureSwab, Becton-Dickinson Co, Franklin Lakes, NJ.
- d. Whirl-Pak bags, Nasco, Fort Atkinson, Wis.
- e. Preston *Campylobacter* selective supplement, Oxoid Ltd, Cambridge, England.
- f. *Campylobacter* growth supplement, Oxoid Ltd, Cambridge, England.
- g. OptiMaxPlus, BioGenex, San Ramon, Calif.
- h. MultiLink, BioGenex, San Ramon, Calif.
- i. Zymed, Invitrogen, Carlsbad, Calif.
- j. NovaRED, Vector, Burlingame, Calif.
- k. Thermo Scientific, Pittsburgh, Pa.
- l. SAS, version 9.1, SAS Institute Inc, Cary, NC.

## References

1. Skirrow MB. Diseases due to *Campylobacter*, *Helicobacter* and related bacteria. *J Comp Pathol* 1994;111:113-149.
2. USDA. Part II: reference of sheep health in the United States. Fort Collins, Colo: USDA, APHIS, Veterinary Services, National Animal Health Monitoring System, 2001.
3. Kirkbride CA. Diagnoses in 1,784 ovine abortions and stillbirths. *J Vet Diagn Invest* 1993;5:398-402.
4. Diker KS, Istanbuluoglu E. Ovine abortion associated with *Campylobacter jejuni*. *Vet Rec* 1986;118:307.
5. Diker KS, Sahal M, Aydin N. Ovine abortion associated with *Campylobacter coli*. *Vet Rec* 1988;122:87.
6. Varga J, Mézes B, Fodor L, et al. Serogroups of *Campylobacter fetus* and *Campylobacter jejuni* isolated in cases of ovine abortion. *Zentralbl Veterinarmed B* 1990;37:148-152.
7. Delong WJ, Jaworski MD, Ward AC. Antigenic and restriction



- enzyme analysis of *Campylobacter* spp associated with abortion in sheep. *Am J Vet Res* 1996;57:163–167.
8. Sahin O, Plummer PJ, Jordan DM, et al. Emergence of a tetracycline-resistant *Campylobacter jejuni* clone associated with outbreaks of ovine abortion in the United States. *J Clin Microbiol* 2008;46:1663–1671.
9. Mannering SA, Marchant RM, Middelberg A, et al. Pulsed-field gel electrophoresis typing of *Campylobacter fetus* subsp. *fetus* from sheep abortions in the Hawke's Bay region of New Zealand. *N Z Vet J* 2003;51:33–37.
10. Mannering SA, West DM, Fenwick SG, et al. Pulsed-field gel electrophoresis typing of *Campylobacter fetus* subsp. *fetus* isolated from sheep abortions in New Zealand. *N Z Vet J* 2004;52:358–363.
11. Mannering SA, West DM, Fenwick SG, et al. Pulsed-field gel electrophoresis of *Campylobacter jejuni* sheep abortion isolates. *Vet Microbiol* 2006;115:237–242.
12. SultanDosa AB, Bryner JH, Foley JW. Pathogenicity of *Campylobacter jejuni* and *Campylobacter coli* strains in the pregnant guinea pig model. *Am J Vet Res* 1983;44:2175–2178.
13. Taylor DE, Bryner JH. Plasmid content and pathogenicity of *Campylobacter jejuni* and *Campylobacter coli* strains in the pregnant guinea pig model. *Am J Vet Res* 1984;45:2201–2202.
14. Bryner JH, Foley JW, Hubbert WT, et al. Pregnant guinea pig model for testing efficacy of *Campylobacter fetus* vaccines. *Am J Vet Res* 1978;39:119–121.
15. Bryner JH, Foley JW, Thompson K. Comparative efficacy of ten commercial *Campylobacter fetus* vaccines in the pregnant guinea pig: challenge with *Campylobacter fetus* serotype A. *Am J Vet Res* 1979;40:433–435.
16. Bryner JH, Firehammer BD, Wesley IV. Vaccination of pregnant guinea pigs with *Campylobacter fetus*: effects of antigen dose, *Campylobacter* strain, and adjuvant type. *Am J Vet Res* 1988;49:449–455.
17. Coid CR, O'Sullivan AM, Doré CJ. Variations in the virulence, for pregnant guinea pigs, of campylobacters isolated from man. *J Med Microbiol* 1987;23:187–189.
18. Parkhill J, Wren BW, Mungall K, et al. The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hyper-variable sequences. *Nature* 2000;403:665–668.
19. Bell JA, Manning DD. Reproductive failure in mink and ferrets after intravenous or oral inoculation of *Campylobacter jejuni*. *Can J Vet Res* 1990;54:432–437.
20. Zhang Q, Meitzler JC, Huang S, et al. Sequence polymorphism, predicted secondary structures, and surface-exposed conformational epitopes of *Campylobacter* major outer membrane protein. *Infect Immun* 2000;68:5679–5689.
21. Coid CR, Fox H. Short review: campylobacters as placental pathogens. *Placenta* 1983;4:295–305.
22. Smith JL. *Campylobacter jejuni* infection during pregnancy: long-term consequences of associated bacteremia, Guillain-Barre syndrome, and reactive arthritis. *J Food Prot* 2002;65:696–708.
23. Simor AE, Karmali MA, Jadavji T, et al. Abortion and perinatal sepsis associated with campylobacter infection. *Rev Infect Dis* 1986;8:397–402.
24. Ertaş HB, Özbey G, Kiliç A, et al. Isolation of *Campylobacter jejuni* and *Campylobacter coli* from the gall bladder samples of sheep and identification by polymerase chain reaction. *J Vet Med B Infect Dis Vet Public Health* 2003;50:294–297.
25. Açık MN, Çetinkaya B. Heterogeneity of *Campylobacter jejuni* and *Campylobacter coli* strains from healthy sheep. *Vet Microbiol* 2006;115:370–375.
26. Poly F, Guerry P. Pathogenesis of *Campylobacter*. *Curr Opin Gastroenterol* 2008;24:27–31.
27. Young KT, Davis LM, Dirita VJ. *Campylobacter jejuni*: molecular biology and pathogenesis. *Nat Rev Microbiol* 2007;5:665–679.
28. Ladrón de Guevara C, Gonzalez J, Peña P. Bacteraemia caused by *Campylobacter* spp. *J Clin Pathol* 1994;47:174–175.
29. Walker RI, Caldwell MB, Lee EC, et al. Pathophysiology of *Campylobacter* enteritis. *Microbiol Rev* 1986;50:81–94.
30. Blaser MJ, Perez GP, Smith PF, et al. Extraintestinal *Campylobacter jejuni* and *Campylobacter coli* infections: host factors and strain characteristics. *J Infect Dis* 1986;153:552–559.
31. Skirrow MB, Jones DM, Sutcliffe E, et al. *Campylobacter* bacteraemia in England and Wales, 1981–91. *Epidemiol Infect* 1993;110:567–573.
32. Korman TM, Varley CC, Spelman DW. Acute hepatitis associated with *Campylobacter jejuni* bacteraemia. *Eur J Clin Microbiol Infect Dis* 1997;16:678–681.
33. Reddy KR, Farnum JB, Thomas E. Acute hepatitis associated with campylobacter colitis. *J Clin Gastroenterol* 1983;5:259–262.
34. Cullen JM. Liver, biliary system and exocrine pancreas. In: McGavin MD, Zachary JF, eds. *Pathologic basis of veterinary disease*. 4th ed. St Louis: Elsevier Mosby, 2007;434–435.
35. Campero CM, Anderson ML, Walker RL, et al. Immunohistochemical identification of *Campylobacter fetus* in natural cases of bovine and ovine abortions. *J Vet Med B Infect Dis Vet Public Health* 2005;52:138–141.
36. van Spreuwel JP, Duursma GC, Meijer CJ, et al. *Campylobacter* colitis: histological immunohistochemical and ultrastructural findings. *Gut* 1985;26:945–951.
37. Davies J, Dempsey EW, Amoroso EC. The subplacenta of the guinea-pig: development, histology and histochemistry. *J Anat* 1961;95:457–473.
38. Mess A. The guinea pig placenta: model of placental growth dynamics. *Placenta* 2007;28:812–815.
39. Carter AM. Animal models of human placentation—a review. *Placenta* 2007;28:S41–S47.
40. Hedstrom OR, Sonn RJ, Lassen ED, et al. Pathology of *Campylobacter jejuni* abortion in sheep. *Vet Pathol* 1987;24:419–426.